VOLUME 10 NOMOR 1 JUNI 2023 **ISSN 2548 – 611X**

JURNAL
BIOTEKNOLOGI & BIOSAINS INDONESIA

Homepage Jurnal: http://ejurnal.bppt.go.id/index.php/JBBI

MUTATION DETECTION OF MULTIDRUG-RESISTANT TUBERCULOSIS BY RT-PCR METHOD AS THE DIAGNOSTIC TOOL FOR MDR-TB

Deteksi Mutasi Multidrug-Resistant Tuberculosis dengan Metode RT-PCR sebagai Diagnostik MDR-TB

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ABSTRACT

Eight percent of tuberculosis (TB) cases worldwide are resistant to rifampicin, with mutations occurring in the rpoB and katG genes. It is necessary to develop a specific multidrug-resistant (MDR) diagnostic technique using the RT-PCR method in Indonesia to aid in rapid and accurate diagnosis. In-silico testing using SnapGene software resulted in the design of DNA primers for the katG and rpoB genes, plasmids, and specific probes. This study employed a cross-sectional design using 30 non-MDR-TB and MDR-TB samples from RSUD Sitanala, Tangerang Banten, which were tested for amplification of the katG and rpoB genes using Sybr green RT-PCR. Validity testing was conducted using specific probes for the katG and rpoB genes. The amplification results showed that MDR-TB samples and MDR-TB plasmids required a longer time compared to non-MDR-TB samples and non-MDR-TB plasmids. The Quantification Cycle (Cq) value in non-MDR-TB samples was lower than the Cq value in MDR-TB samples. A t-test revealed a significant difference in Cq values of the rpoB and katG genes between MDR-TB and non-MDR-TB patients (p-value < 0.005). These differences in Cq values indicate that the findings of this study can serve as an initial reference for the development of an RT-PCR-based diagnostic kit for MDR-TB.

Keywords: In-silico, katG gene, MDR-TB, rpoB gene, RT-PCR

ABSTRAK

Sekitar 8% Tuberculosis (TB) di seluruh dunia resisten terhadap rifampisin, dan mutasi terjadi pada gen rpoB dan katG. Perlu dikembangkan teknik diagnosis multidrug resisten (MDR) dengan metode RT-PCR yang spesifik di Indonesia untuk membantu penegakan diagnosis secara cepat dan tepat. Uji in-silico menggunakan perangkat lunak SnapGene menghasilkan desain primer DNA gen katG dan rpoB, plasmid, dan probe spesifik. Penelitian ini menggunakan uji potong lintang pada 30 sampel non-MDR-TB dan MDR-TB dari RSUD Sitanala, Tangerang Banten yang diujikan dengan amplifikasi gen katG dan rpoB dengan menggunakan Sybr green RT-PCR. Uji validitas menggunakan probe gen katG dan rpoB yang spesifik. Hasil amplifikasi memperlihatkan sampel MDR-TB dan plasmid MDR-TB lebih lambat dari sampel dan plasmid non-MDR-TB. Nilai Quantification Cycle (Cq) pada TB non-MDR lebih rendah dari nilai cq MDR-TB. T-test menunjukkan perbedaan nilai Cq gen rpoB dan katG pada pasien MDR-TB dan non-MDR-TB (p-value <0,005). Adanya perbedaan nilai cq tersebut menunjukkan hasil penelitian sebagai acuan awal dalam pembuatan kit diagnostik MDR-TB dengan metode RT-PCR.

Kata kunci: Gen katG, gen rpoB, in-silico, MDR-TB, RT-PCR

Received: *19 March 2023* ***Accepted:*** *13 June 2023* ***Published:*** *30 June 2023*

INTRODUCTION

Tuberculosis (TB) is a dangerous infectious disease, and approximately 140,000 deaths occur each year (Falzon et al. 2015, Lestari et al. 2020). In 2013, Indonesia was ranked as the fourth highest country in the world with TB-related issues (Reviono et al. 2014, Falzon et al. 2015, Susanty et al. 2016, Maladan et al. 2021b). Many individuals in Indonesia do not adhere to antibiotic treatment (Table 1). Globally, about 8% of TB patients have rifampicin-resistant TB (Moure et al. 2011, Reviono et al. 2014, Maladan et al. 2020, Jang and Chung 2020, Yuliwulandari et al. 2021). The estimated number of TB-resistant cases is 12,000, originating from 2.4% of new cases and 13% of re-treatment cases. Indonesia still ranks third in the world in terms of new tuberculosis cases, posing a significant challenge that requires attention from all stakeholders due to the high burden of morbidity and mortality it carries (Kementrian Kesehatan RI 2019).

Several studies have reported that more than 90% of rifampicin-resistant TB patients are also resistant to isoniazid. Rifampicin inhibits bacterial growth by strongly binding to bacterial DNA-dependent RNA polymerase, thereby inhibiting bacterial RNA synthesis. Thus, resistance to rifampicin can serve as a surrogate marker for Multidrug Resistance (MDR-TB) (Falzon et al. 2015, Susanty et al. 2016, Reichmuth et al. 2020, Maladan et al. 2021b, Yuliwulandari et al. 2021).

Resistance to rifampicin in *Mycobacterium tuberculosis* is primarily caused by mutations in the rpoB gene, which encodes the β subunit of RNA polymerase in 81 hot spot regions (Lange et al. 2014). This β subunit, encoded by the rpoB gene, binds to nucleotides and transcription inhibitors such as the antibiotic rifampicin (Reviono et al. 2014). Whole Genome Sequencing revealed a mutation in the Serine codon at position 513 of the rpoB gene and position 135 of the katG gene (Table 2) (Tania et al. 2020, Maladan et al. 2021a).

The cause of this resistance is the substitution of the amino acid Serine (TCG) with Leucine (TTG) at codon 531 (Ser531Leu). Concurrently, the cause of isoniazid resistance is related to gene mutations because the mechanism of action of antibiotics for treating TB targets specific genes in *M. tuberculosis* bacteria, thereby inhibiting vital biochemical processes necessary for the bacteria's survival. Some of the genes involved in isoniazid resistance include katG, inhA, kasA, and aphC, with the most prevalent mutations found in the katG15 gene (Maladan et al. 2021a).

Identifying these TB patients and providing timely and accurate diagnosis and

Table 1. Number of Indonesian genomes identifying antibiotic resistance (Tania et al. 2020, Maladan et al. 2021)

Origin of the samples	Number	Resisten to rifamicin	Resisten to isoniazid	MDR
Bandung	51	10	29	8
Bogor	20	21	17	10
Jakarta	10	6	4	4
Papua	19	16	15	15
Total	**100**	**53**	**65**	**37**

Table 2. Codons in the mutated katG and rpoB genes in MDR-TB patients (Tania et al. 2020)

SNP Mutations of the katG gene	Number of samples	SNP mutation of the rpoB gene	Number of samples
Ser315Thr	42	Ser450Leu	34
Ser140Asn	2	His445Arg	4
Trp191Arg	2	His445Tyr	3
Gly279Asp	1	Gln432Leu	2
Gln 127Pro	1	Ser450Trp	2
Ser315Asn	1	His445Asp	2
Ala379Val	2	Asp435Tyr	3
Ser315Met	1	Asp435Val	2
		His445Cys	1
TOTAL	52*		53

treatment according to international standards are crucial for disease control (Narasimooloo and Ross 2012, Reviono et al. 2014, Rao et al. 2016, Lestari et al. 2020). The gold standard for diagnosing TB is examining solid or liquid cultures, but this technique is time-consuming, taking approximately 6-9 weeks (Berkhout and Haasnoot 2009, Hewajuli and Dharmayanti 2014, Nguyen et al. 2019). One of the molecular methods for detecting *M. tuberculosis* mutations is Real-Time Polymerase Chain Reaction (qPCR) (Reviono et al. 2014, Rao et al. 2016, Terranova et al. 2018, Ember et al. 2022). Nucleic acid-based testing using real-time or quantitative PCR (RT-PCR or qPCR) enables the detection of mutations associated with anti-tuberculosis drug resistance (Hewajuli and Dharmayanti 2014, Dramé et al. 2020, Aoki et al. 2021).

The purpose of this study is to establish a method for detecting gene mutations in MDR-TB patients using the RT-PCR method. RT-PCR tests are urgently needed to identify the latest gene mutations in the *M. tuberculosis* bacterium, ensuring effective, efficient, and accurate test results (Tahamtan and Ardebili 2020, Ember et al. 2022). This test will significantly assist in rapid diagnostic enforcement and help optimize the use of RT-PCR laboratories established throughout Indonesia for Covid-19 testing, which can be converted for TB detection tests.

MATERIALS AND METHODS

Location and time

The Molecular Biology laboratory at Esa Unggul University, Jakarta Barat, and the BioSafety Laboratory level 2 (BSL-2) of Vaccine and Drug Research Center, National Innovation Research Agency (BRIN), Serpong, were the research sites from June to December 2022.

Materials

A cross-sectional research design was employed. A total of 30 samples of non-MDR-TB and MDR-TB patients were obtained from Sitanala Hospital, Tangerang Banten (RJQM+2R Karang Sari, Tangerang City, Banten). The materials for DNA isolation included the bead Pathogen Nucleic Acid Purification Kit (Cat No. NAP40024-01, Biosearch Technologies), binding buffer, protease solution, lysis buffer, buffer BN1, buffer TN 1, buffer solution TN2, and Adenosine Mono Phosphate (AMP) elution buffer. The materials for the RT-PCR test included 2X SYBR Green PCR Mastermix (SolarBio, SR1110), DNA primers (rpoB, katG, and IS6110 as a housekeeping gene), ddH2O, and the design of Single nucleotide polymorphism (SNP) specific probes of rpoB and katG genes. Additionally, plasmid design with the insertion of the rpoB and katG genes was prepared. All the primers, probes, and plasmids were specifically designed for non-MDR-TB.

Method

The Whole Genome Sequencing (WGS) data of *M. tuberculosis* and specific SNP information in Indonesia were obtained from the National Centre for Biotechnology Information (NCBI) and literature sources (Table 2) (Tania et al. 2020). The TB-Profiler server was utilized for resistance identification. Reports and BAM files were downloaded for analysis using Unipro Ugene ver 44.0 and Snapgene ver 6.1.0 software. The distribution and frequency of mutations at each SNP point were collected to determine candidate genes and target mutations (Yılmaz et al. 2021).

The design of SNP-specific primers and probes in the rpoB and katG genes was conducted. The design included the adaptation of Locked Nuckeid Acid (LNA) probes to strengthen the probe bond with the template. The probe sequences of 13 bp each on the LNA monomer were added to the SNP base, flanked by two bases. Consensus sequences for the rpoB and katG genes were obtained from WGS analysis. The primary candidates and probes were manually determined using Snapgene ver 6.1.0 software, and the design results were reviewed with the NetPrimer server. Specificity validation was performed using the primary Basic Local Alignment Search Tool (BLAST) server. In Silico melting curve analysis was conducted using the u-Melt Quartz server, and In Silico PCR was performed using Snapgene ver 6.1.0. Silico plasmid construction with the insertion of In Silico PCR results for control design was achieved using Snapgene ver 6.1.0. Plasmid

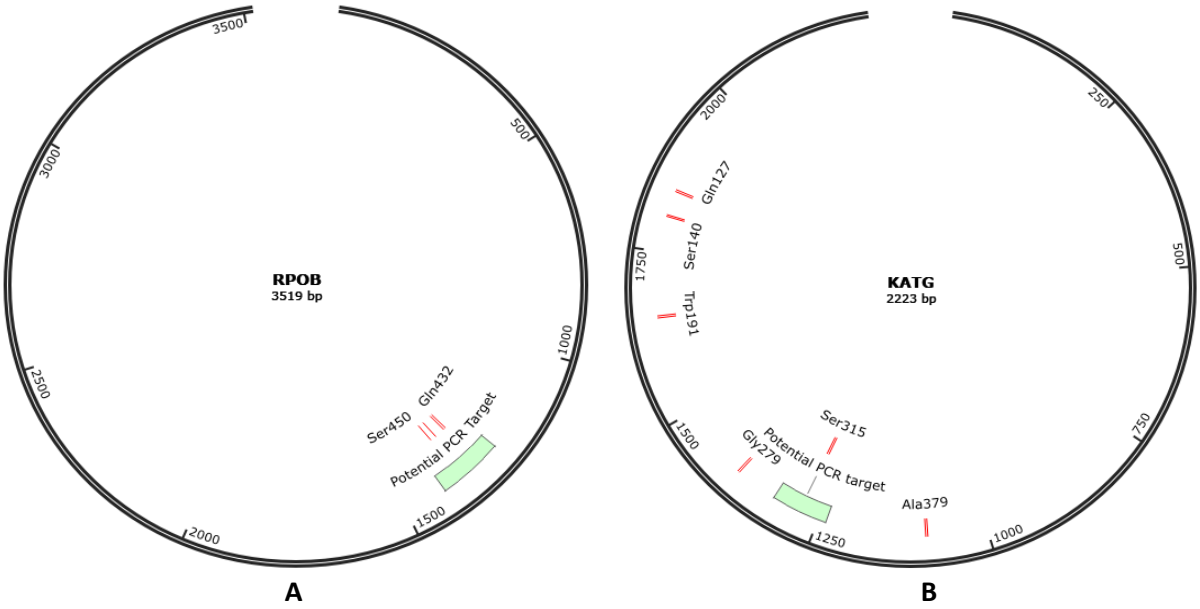

Figure 1. Visualization and annotation of the rpoB gene (A) and KatG genes (B) on plasmids using SnapGene software

construction results were annotated using Snapgene ver 6.1.0 (Figure 1) (Moure et al. 2011, Pandey et al. 2017).

Ethical clearance

Research ethics approval was obtained from the ESA Unggul University Code of Ethics Enforcement Council Research Ethics Commission with the number 0922-08.040/DPKE-KEP/FINAL-EA/UEU/VIII/202.

DNA isolation

DNA isolation was performed using the Sbeadextm Pathogen Nucleic Acid Purification Kit (NAP40-024-01). Personal Protective Equipment (PPE) for BSL 2 was used before isolating *M. tuberculosis* DNA. In the first step, a pre-mix buffer was prepared by adding 20 µL sbeadex particles and 160 µL binding buffer into a 1.5 mL tube, followed by homogenization. Next, a new 1.5 mL tube was prepared, and 20 µL of protease solution, 100 µL of sputum (phlegm) sample, and 100 µL of lysis buffer were added into the tube. The mixture was homogenized, and then incubated at 55°C for 10 minutes.

Subsequently, 180 µL of the premix prepared earlier was added to the incubated and homogenized tube. The tube was incubated for 10 minutes at room temperature with constant shaking. A magnetic rack was prepared, and the tube containing the mixture after incubation was placed on the magnetic rack for 2 minutes, allowing the magnetic particle solution to adhere to the tube wall. In the next step, the tube was removed from the magnetic rack, 400 µL of wash buffer BN1 was added, and the mixture was homogenized. The tube was then incubated for 5 minutes at room temperature with constant shaking. The magnetic rack was used again, and the tube containing the mixture after incubation was placed on the rack for 2 minutes to allow the magnetic particle solution to adhere to the tube wall. The supernatant was discarded.

The tube was removed from the magnetic rack, 400 µL of wash buffer TN1 was added, and the mixture was homogenized. The tube was incubated for 5 minutes at room temperature with constant shaking. The magnetic rack was used once more, and the tube containing the mixture after incubation was placed on the rack for 2 minutes to allow the magnetic particle solution to adhere to the tube wall. The supernatant was discarded.

Continuing with the washing stage, the tube was removed from the magnetic rack, 400 µL of wash buffer TN2 was added, and the mixture was homogenized. The tube was incubated for 5 minutes at room temperature with constant shaking. The magnetic rack was used again, and the tube containing the

mixture after incubation was placed on the rack for 2 minutes to allow the magnetic particle solution to adhere to the tube wall. The supernatant was discarded.

The final step involved adding 100 µL of AMP elution buffer to obtain pure DNA. The tube was then incubated for 10 minutes at 60°C with periodic shaking. After 10 minutes, the tube was placed onto the magnetic rack until the magnetic particles adhered to the entire tube wall. The supernatant containing pure DNA was collected and transferred to a new tube.

The primers and probes were diluted according to the available protocol, with an initial dilution of (1:9). The purpose of the dilution was to measure the concentration using the Nanodrop tool. For template DNA, no prior dilution was performed. The computer and Nanodrop device were turned on, and the application for concentration measurement was opened. Primer and probe concentrations were measured using ssDNA measurements, while template DNA concentration was measured using dsDNA measurements. A blank measurement was conducted by dropping 2 µL onto the microplate reader. The microplate reader was wiped with a tissue after each sample change. The volume of each sample dripped onto the microplate reader was 2 µL.

Plasmid preparation

The plasmid, which was previously designed with the insertion of rpoB and katG genes, was isolated using the manual Alkaline Lysis method at the Badan Riset Inovasi Nasional (BRIN). The concentration of the plasmid was measured using the Infinite M200 Pro NanoQuant (Medquest) and adjusted for qPCR optimization.

Quantitative PCR optimization

The 1× running qPCR reaction and qPCR program settings were adapted from the standard protocol provided in the SensiFAST High-Resolution Melting (HRM) Kit (BIO-32020). The qPCR optimization was carried out with a temperature gradient of 60-65 °C to determine the optimal annealing temperature based on the obtained Cycle threshold (Ct) value. Primary concentration optimization was performed using different concentrations: 100 nM, 200 nM, 300 nM, 400 nM, and 500 nM. This optimization was conducted to determine the optimal primer concentration for each primer pair.

Statistical test

The statistical tests used in this study were the homogeneity test and the unpaired t-test for comparison between the MDR-TB and non-MDR-TB groups.

RESULTS AND DISCUSSION

The results of the DNA primer design of the rpoB gene obtained a base length of 123 base pairs (bp) with guanine-cytosine (GC) base and temperature melting (Tm) values that met the requirements of DNA primers (Table 3).

The results of the plasmid design with the insertion of the rpoB gene are as follows:
5’ - CCGGTGGTCGCCGCGATCAAGGAGT TCTTCGGCACCAGCCAGCTGAGCCAATT CATGGACCAGAACAACCCGCTGTCGGG GTTGACC**CAC**AAGCGCCGACTGT**CG**GC GCTGGGGCCCGGCGGTCTGTCACGTGA GCGTGCCGGGCTGGAGGTCCG - 3'

The CAC and CG sequences are the start and end of the rpoB gene sequence inserted into the plasmid with the SNP mutation on Ser450Leu (green color). The rpoB primer DNA design results obtained a base length of 123 bp with GC and Tm values that met the requirements of DNA primers (Table 3).

The results of the plasmid design with the insertion of the katG gene are described in the following base sequence:
5’-
GCAGATGGGCTTGGGCTGGAAGAGCT

Table 3. Results of DNA primer gene design and rpoB probe

Name	Sequences	length	Tm (°C)	GC (%)	Product size
rpoB-F-UEU	ATCAAGGAGTTCTTCGGCACC	21	60,67	52,38	123 bp
rpoB-R-UEU	ACGCTCACGTGACAGACCG	19	59,71	63,16	
Probe*	HEX-agcgcCGAcagtc-BHQ1	13	41,74	69,23	

*The designed probe is adapted to make LNA-Probes with the addition of monomers.

CGTATGGCACCGGAACCGGTAAGGACG
CGATCACCAGCGGCATCGAGGTCGTAT
GGACGAACACCCCGACGAAATGGGACA
ACAGTTTCCTCGAGATCCTGTACGGCTA
CGA-3'

The CG and CG sequences (red color) are the start and end of the rpoB gene sequence inserted into the plasmid with the SNP mutation on Ser315Thr (green color) (Table 4). The template for plasmid is:

5'-CTGCGCGATGGCGAACTCAAGGAGCA
CATCAGCCGCGTCCACGCCGCCAACTAC
GGTGTTTACGGTGCCCGCAAAGTGTGGC
TAACCCTGAACCGTGAGGGCATCGAGGT
GGCCAGATGCACCGTCGAACGGCTGAT
GACCAAACTCGGCCTGTCC-3'

Single nucleotide polymorphism (SNP) resistance to rifampicin and isoniazid occurs in the rpoB and katG genes (Maladan et al. 2020). Several SNP points occur, but based on the TB-Profiler analysis, the most common SNPs are at codon 450 for the rpoB gene and codon 315 for the katG gene, as the target genes for the design of primers and specific probes that will differentiate between multidrug-resistant tuberculosis (MDR-TB) and non-MDR. Furthermore, the rpoB and katG genes from several whole-genome sequencing (WGS) results in each region were aligned and analyzed with Snapgene software. The design results using the Snapgene plasmid software are shown in Figure 1 below.

In this study, an RT-PCR MDR-TB diagnostic test was developed, preceded by literature data and an in-silico trial. In this literature test, 100 genomes of *M. tuberculosis* were obtained, with 81 genomes coming from West Java and 19 from Papua. Fifty-three samples were resistant to rifampicin, and 65 were resistant to isoniazid. Thirty-seven were resistant to isoniazid and rifampicin, categorized as MDR-TB (Table 2) (Falzon et al. 2015, Tania et al. 2020).

Single nucleotide polymorphisms of the rpoB and katG genes cause resistance to rifampicin and isoniazid (Reviono et al. 2014). Plasmid, probe, and primer design using Snapgene software and the BLAST method resulted in the insertion of genes into plasmids with codon precision of 450 for the rpoB gene and 135 for the katG gene. Several SNP points occur based on analysis with the TB-Profiler. It can be seen that the most common SNP is at codon 450 for the rpoB gene and codon 315 for the katG gene (Artauli et al. 2021, Maladan et al. 2021a, Tania et al. 2020).

SNP mutations in the katG and rpoB genes are dominant in MDR-TB patients. The mutations that occur are point mutations from C to G in the rpoB gene codon and T to A in the katG gene codon (Falzon et al. 2015, Maladan et al. 2021a). This change in codon bases in *M. tuberculosis* makes it challenging to detect and amplify when attaching to DNA primers (Reviono et al. 2014, Gill et al. 2022). Therefore, the rpoB and katG gene plasmid designs were used as controls, and the primer and probe designs were intended for detection in MDR-TB patient samples.

Based on the results of SNP identification and determination of the consensus sequence from the previous analysis, the consensus sequence of the rpoB and katG genes was used as a template for conducting primer and probe design. The targeted mutations in each gene have been adapted to modifications common in Indonesia and Asian countries with high

Table 4. Results of DNA primer gene design and rpoB probe

Name	Sequences	length	Tm (°C)	GC (%)	Product size
katG-F-UEU	AGCTCGTATGGCACCGGAA	19	61,23	57,89	89bp
katG-R-UEU	CTGTTGTCCCATTTCGTCGG	20	60,98	55,0	
Probe*	FAM-tcaccAGCggcat-BHQ1	13	43,08	61,54	

*The designed probe is adapted to make LNA probes with the addition of basic monomers.

Table 5. Universal gene primer design results - IS6110

Name	Sequences	Length	Tm (°C)	GC (%)	Product size
IS6110-F	CGAACTCAAGGAGCACATCAG	21	62.6	50.0	134bp
IS6110-R	TCAGGGTTAGCCACACTTTG	20	62.1	52.6	
Probe*	CGGGCACCGTAAACACCGTAGT	22	68.1	59	

cases, such as India, Thailand, and China (Falzon et al. 2015, Gill et al. 2022). The primers designed have been adapted for multiplex high-resolution melting (HRM) analysis applications. However, in-silico experiments showed a possible cross-dimer between rpoB-F and Uni-R, self-dimer on rpoB-R, and katG-F. The results of the primer design in-silico optimization test showed that the Tm for the katG gene did not change at 0.25 increments because the SNP that occurred was G > C, so it did not change the %GC, which affected the melting temperature (Yılmaz et al. 2021). Ruesen (2019) showed a single mutation in several gene codons in patients with MDR-TB, making it difficult to amplify the gene. The difference in gene mutations in COVID-19 patients who experience deletions makes amplifying genes easier (Ruesen 2019).

The optimization results from testing for the annealing temperature of the DNA primers for the rpoB and katG genes in the RT-PCR test were 58 °C. The results of the RT-PCR test using patient samples, probes, non-MDR-TB plasmids, and MDR-TB plasmids as controls showed amplification of the katG and rpoB genes in TB and MDR-TB patients. The validation test for inserting a gene into a plasmid was carried out using a gene amplification test using a probe designed to recognize the katG and rpoB genes. The test results showed success in amplifying the two genes correctly (Figure 2 and Figure 3).

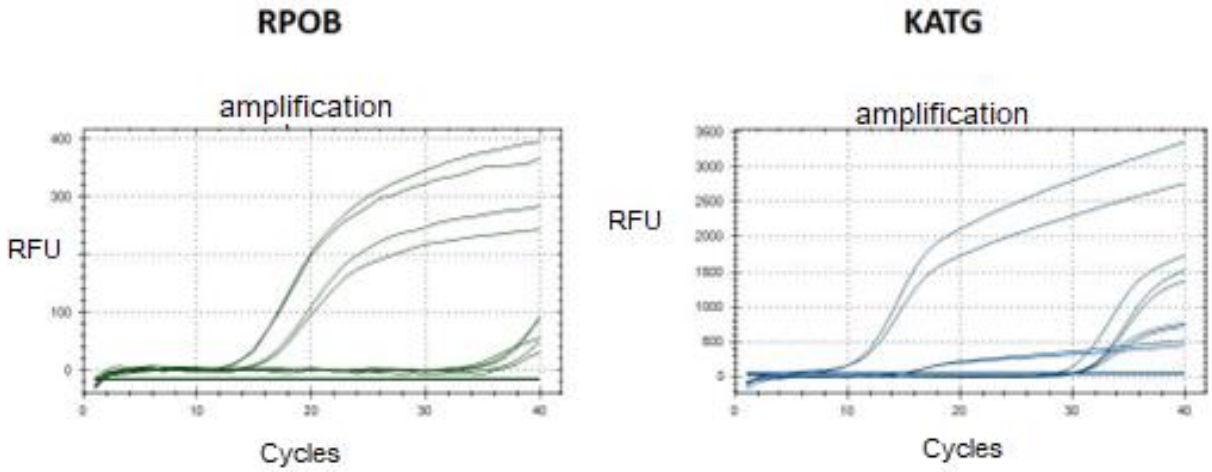

Figure 2. Results of the rpoB and katG gene amplification test using a probe to validate the genes on the plasmid

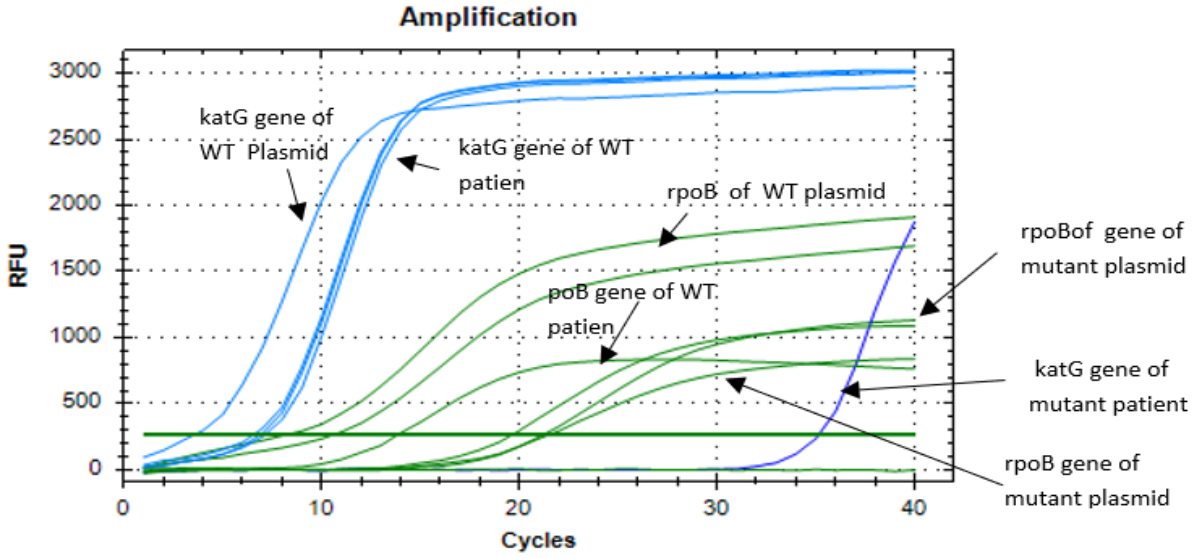

Figure 3. Results of the rpoB and katG gene amplification test using a probe to validate the gene on the plasmid (The graphs for katG and rpoB are shown by the blue and green lines, respectively).

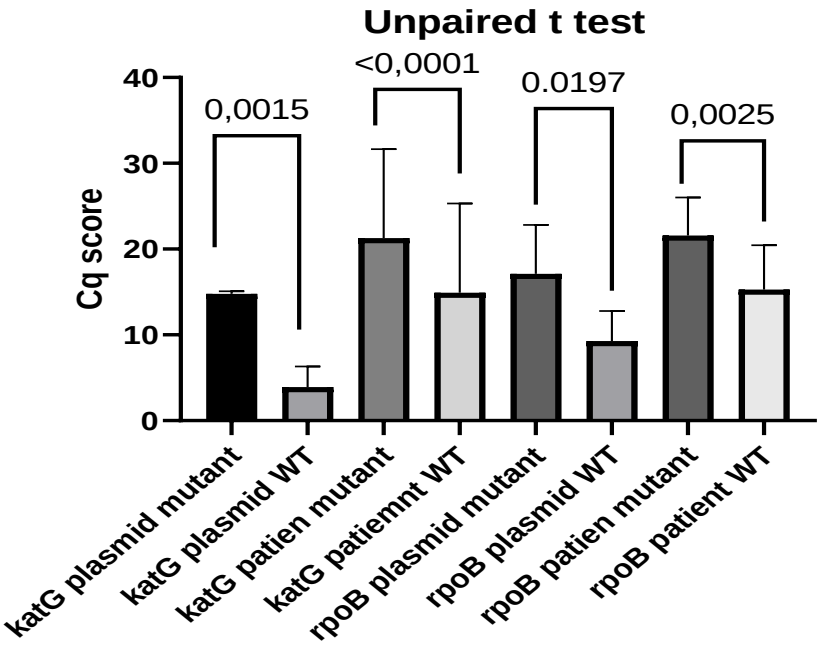

Figure 4. Comparative results of paired T-Test graph showing significant difference in Cq scores ($p < 0.05$) between the Wild-Type (WT; non-MDR-TB) group and each treatment group.

Figure 5. Sputum sample from MDR-TB patient (black circle)

The RT-PCR test results for MDR-TB and non-MDR-TB patients using DNA primers, probes, and plasmids as controls showed successful amplification of the katG and rpoB genes. The success of rpoB and katG gene amplification shows that the gene primer and probe designs recognized the gene sequences in non-MDR-TB and MDR-TB patients. A patient's DNA amplification results can describe the patient's condition, whether classified as MDR-TB or non-MDR-TB. Patients classified as non-MDR-TB appear to have the curve amplified earlier, and non-MDR-TB plasmids are amplified earlier. Meanwhile, patients will be confirmed as MDR-TB if the amplified curve is slower than non-MDR-TB.

Amplification of MDR-TB samples and MDR-TB plasmids was delayed compared to non-MDR-TB samples and plasmids. The probe is designed for non-MDR-TB plasmids, so the first amplification will occur in non-MDR-TB samples and be followed by mutant models. Amplification in non-MDR-TB patients and non-MDR-TB plasmids occurred earlier than in MDR-TB samples and MDR-TB plasmids, indicating that the

Quantification Cycle (Cq) value in non-MDR-TB is lower than the MDR-TB Cq value.

The statistical test results showed that the data were normally distributed with a p-value > 0.005. The comparison test results between MDR-TB and non-MDR-TB data showed a significant difference in the Cq value resulting from the amplification of mutations in the katG gene and the rpoB gene, with a p-value < 0.005 (Figure 4). There were significant differences in amplification results between MDR-TB and non-MDR-TB plasmids and between non-MDR-TB and MDR-TB patient samples.

The statistical test results showed a significant difference in the Cq value of the amplification of the katG gene in MDR-TB plasmids and non-MDR-TB plasmids. Similarly, there was a significant difference in the Cq value in the results of rpoB gene amplification in non-MDR-TB and MDR-TB plasmids, with $p < 0.05$. The same goes for the amplification test of the katG gene and the rpoB gene in MDR-TB and non-MDR-TB patients, which showed a difference in the Cq value for rpoB and katG in MDR-TB and non-MDR-TB patients, with a p-value < 0.05. The results of this study can be further tested for validity as a potential RT-PCR diagnostic test for MDR-TB patients.

CONCLUSION

The results of in silico DNA primer, probe, and plasmid designs from WGS data obtained from patients spread across Bogor, Jakarta, Bandung, and Papua were successful in amplifying primers, probes, and plasmids in both MDR-TB and non-MDR-TB patients. Statistical analysis revealed a significant difference in the Cq value between MDR-TB and non-MDR-TB patients. Therefore, these in silico test results can be further validated as a potential RT-PCR diagnostic test for MDR-TB patients.

ACKNOWLEDGMENT

Thanks to Kedaireka for the grant with research contract 367/E1.1/KS.06.02/2022. We would also like to express our gratitude to PT Ecosains for their support as a research partner. Additionally, we extend our thanks to the Vaccine and Drug Research Center of BRN for their collaboration in this research and for providing us with laboratory facilities. We are grateful to the Biotechnology Study Program team and the leaders and staff of the University of Excellence for their valuable assistance and cooperation.

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